a percent, apparently indicating that a very small change in composition leads to a significant change in the rate of exocytosis.

WORKSHOP 3: Applications of Small Angle X-Ray Scattering

197-Wkshp
X-Ray Scattering (SAXS) Combined with Crystallography and Computation: Defining Accurate Macromolecular Structures, Conformations and Assemblies in Solution
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Protein, DNA, and RNA shape, assembly and interactions shapes, plus their detailed structural chemistry, encode key information regarding pathway choices and biological outcomes. We are developing SAXS combined with crystallography as a premiere tool for defining macromolecular conformations and connections suitable to join proteins to pathways and at the proteomic scale[1-2]. Crystallography supplies unsurpassed structural detail for mechanistic analyses; however, it is restricted to describing conformations of macromolecules within crystal lattices. In principle, SAXS can provide reliable complementary data on small and large macromolecules[3]. In practice, SAXS can be limited by problems in optimizing samples and analyses, which can be reduced or avoided. Our results on dynamic RNA, DNA, and protein complexes show that SAXS has great potential to provide accurate shapes, conformations, and assembly states in solution and inform biological functions in fundamental ways[4-5].
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198-Wkshp
Macromolecular X-Ray Solution Scattering with Third Generation Synchrotron Sources
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One factor driving the resurgence in the popularity of macromolecular small-angle solution scattering has been the availability of new small-angle scattering instruments on third generation synchrotron sources. The high intensity, low background and the high sensitivity detectors available at these facilities has allowed the collection of better signal to noise scattering patterns, over wider ranges of scattering vector, and in less time. The result is more stringent data for modeling efforts using ever more sophisticated codes. In addition, the high fluxes and small beams delivered by these beamlines are ideal for macromolecular folding studies using stopped flow and various continuous flow micro-fluidic mixers. For folding studies, the primary advantage of SAXS over competing solution techniques, most of which are sensitive to local structural changes (such as florescence), is that it provides information on global shape changes. Here I will review a number of these applications that have been done at the BioCAT facility at the Advanced Photon Source, Argonne National Labs as well as future prospects for wider application of these techniques.

199-Wkshp
Structure and Function of Biological Macromolecules in Solution: The Unique Role of Small-Angle Neutron Scattering
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Small-angle neutron scattering (SANS) is a useful tool for investigating the structure of complex biological macromolecules in solution. Neutrons are very sensitive to the scattering from the light elements such as hydrogen, carbon, nitrogen and oxygen which are of central importance to all biological systems. In addition, hydrogen and deuterium have very different neutron scattering strengths so the isotopic substitution of D for H is routinely used to change the scattering from a system without affecting its biochemistry. The concept of SANS from biological macromolecules will be introduced, with an emphasis on how SANS can provide unique insight into macromolecular structure and function. Specific examples will be provided, including the use of D-H substitution and contrast variation to determine the structure of the individual components in a two-component complex.

200-Wkshp
AAA+ ATPase Mechanism
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AAA+ ATPases contribute to nearly all cellular activities. Nature builds these machines as homomeric rings with catalytic sites residing between each protomer. A highly conserved catalytic core is modified with task-specific structural elements to generate a myriad of functions. Structural details about how these motors work are becoming available. Here we describe studies of NtrC1, a member of the NtrC subfamily of AAA+ ATPases that bacteria use to regulate gene expression from the σ54-form of RNA polymearase. In NtrC-like ATPases the specificity motifs are two loops called L1 and L2, with the latter containing a 'GAFTGA' motif for direct binding to σ54 of RNAP. Interaction of the ATPase with the polymerase remodels the sigma factor enabling it to melt promoter DNA so that transcription can begin. We show by SAS that while NtrC1 variant E239A is unable to hydrolyze ATP it undergoes nucleotide-driven conformational changes typical of the wild type protein and stably binds to σ54. An ATP-bound crystal structure revealed for the first time the γ-phosphate interacting with the catalytically crucial R-finger. Comparing the new structure with a prior ADP-bound suggests that R-finger engagement propagates a series of conformational changes on the R-finger side of the protomer/protomer interface, causing the L1-GAFTGA motif to extend several Angstroms above the plane of the ring to bind σ54. Neighbor/neighbor contacts between protomers, notably in a rigid body formed between the bulk of the L1- and L2-loops, suggest that nucleotide binding and subsequent conformational change will be complex, and likely cooperative. To test this hypothesis we took advantage of recent developments at the BioCAT beam line to perform static and time-resolved SAXS experiments to monitor conformational changes as nucleotide occupancy progresses from apo to fully bound state. The results reveal a series of changes with at least one intermediate state.